

Opposite Effects of Dextran Substituted with Sulfhydryls or Mercury on Tumor Growth

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ABSTRACT

Macromolecular dextrans carrying substituents terminated by sulfhydryl groups or terminated by aromatic amines effectively inhibit the growth of a fibrosarcoma and of a mammary adenocarcinoma in a syngeneic mouse model. These compounds have no or very low toxicity to animals and are nontoxic to fibrosarcoma cells *in vitro*. Small-molecular-weight compounds carrying the same substituents as the above dextrans are without any effect on the growth of these tumors. A dextran substituted with mercury-containing side chains is growth promoting for the same fibrosarcoma in mice at doses which are nontoxic for these animals. However, the mercury-containing compound is toxic to fibrosarcoma cells *in vitro*. It is hypothesized that these nonpermeating macromolecules do not directly influence the tumor cells in animals but modulate the natural system of defense against tumors; cells of that system are stimulated or poisoned by the substituted dextrans.

INTRODUCTION

Several natural polysaccharides have antitumor properties (19), e.g., glucan (16), levan (8), zymosan (3), and lentinan (5). Antitumor properties may also be conferred by a suitable chemical substitution on an inactive polysaccharide. Thus, from dextran one may obtain dextran sulfate, which activates macrophages and is an antitumor agent (17); substitution of dextran by phosphate and fatty acid residues achieves a similar potency (18). Alternatively, a polysaccharide may be used as a carrier which slowly releases an antitumor drug, e.g., daunomycin (2). The present study was designed to investigate dextrans with electroneutral substituents, which have the ability to react with sulfhydryl groups. Sulfhydryl and disulfidic groups seem to have importance as elements of cell surface. They were found to be present in increased amounts in transformed cells (10). Furthermore, a number of chemotherapeutic agents react with these groups, e.g., terpene lactones, showdomycin, or acridine derivatives (4, 7). A number of radioprotective and antitumor agents also contain thiol groups free or in blocked forms (1).

MATERIALS AND METHODS

Compounds. Structures of the compounds studied are shown in Chart 1. All were prepared from the dextran fraction with an average molecular weight of 170,000. Macromolecular Compound 1 was prepared by oxidation of dextran with periodic acid, as described previously (14) and

subsequent reduction with sodium borohydride. It differs from the starting dextran only by having some of the glucose units replaced by residues carrying primary alcohol groups. Aniline-dextran 2 was prepared by condensation of the oxidized dextran with 4,4'-diaminodiphenylmethane (Chart 1, Compound 3), and by the reduction of the condensation product (14). Compound 3 was obtained from Eastman Organic Chemicals, Rochester, N. Y. Sulfhydryl-dextrans 4 and 5 were prepared from 2 preparations of aniline-dextran 2, which differed by the degree of substitution; the reaction with *N*-acetylhomocysteine thiolactone was used to introduce the required sulfhydryl groups. Aniline-dextran 2 (2 g) was first dissolved in water (20 ml), and after adding 20 ml of 1 M sodium carbonate buffer, pH 9.7, *N*-acetylhomocysteine thiolactone (1 g) was introduced into the ice-chilled mixture. The solution was left overnight at 4° and then dialyzed successively against water, 0.1% mercaptoethanol, water, and finally 0.9% NaCl solution; all these operations were performed at 4°. Composition of the products obtained was checked by elemental analysis (for nitrogen and sulfur) of the freeze-dried samples. Model Compound 6 represents a part of the side chains of Compounds 4 and 5. It was prepared by the condensation of *N*-acetylhomocysteine thiolactone with 4-aminobenzoic acid. Sodium 4-aminobenzoate (0.9 g) was dissolved in 5 ml 1 M carbonate buffer, pH 9.7; *N*-acetylhomocysteine thiolactone (0.8 g) was then added; and the mixture was left overnight. The solution was brought to pH 1 with hydrochloric acid and extracted 10 times with ethyl acetate. The ethyl acetate extract contained Product 6, the corresponding disulfide, and a small amount of 4-aminobenzoic acid. The solution was left in contact with air in order to oxidize all the sulfhydryl Compound 6 to the corresponding disulfide. The disulfide was easily purified by crystallization from ethyl acetate, m.p. 188-190°.



Calculated: N 9.49, S 10.85

Found: N 9.19, S 11.17

To convert the disulfide into sulfhydryl Compound 6, the following procedure was used. Disulfide (40 mg) was suspended in 10 ml of water and sodium borohydride (10 mg) was added. Conversion to water-soluble sulfhydryl compounds occurred immediately. Compound 7, synthesis of which was previously described (14), contained mercury covalently attached to the polysaccharide. It was tested in the form of cysteine derivative in order to prevent complications because of the low solubility of chloride and phosphate complexes (14).

Animals and Tumors. Animals used were 12- to 14-week-old male mice of the inbred subline C3Hf/Sed (from the C3H/He strain), raised in the pathogen-free environment of

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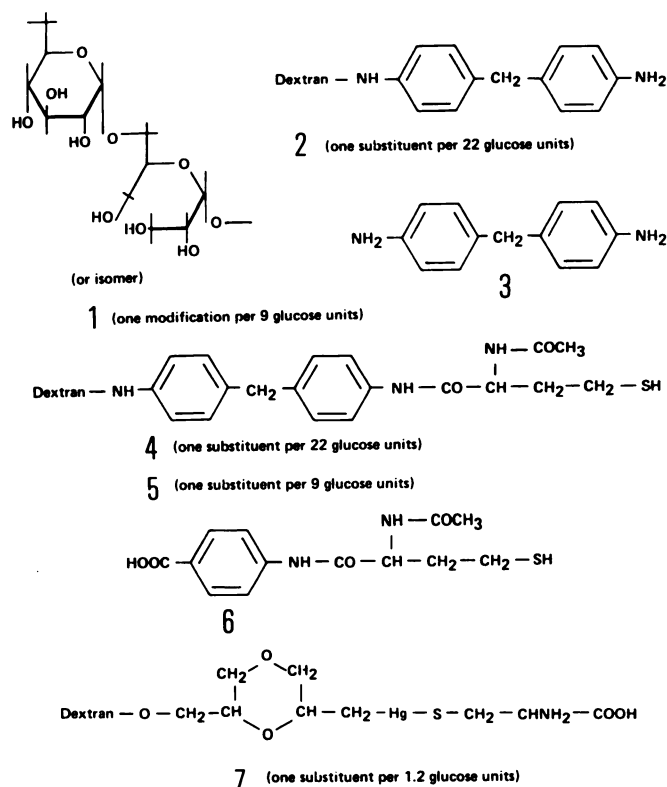


Chart 1. In the substituted dextrans, the substituents are attached directly to carbon atoms of the polysaccharide.

the Pondville animal colony. One of the solid tumors used was a methylcholanthrene-induced fibrosarcoma that was preserved in liquid nitrogen and reintroduced into the syngeneic C3Hf/Sed mice, in which it had been originally produced. When used for assays, this tumor was in its 15th to 20th transplant generation. The other tumor was an adenocarcinoma in the same strain, derived from a spontaneous mammary adenocarcinoma in a C3Hf/Sed mouse. It was used when in the 8th to 12th generation.

Assays. Pieces of 12- to 14-day old healthy tumor were inoculated with trocar (2-mm diameter, pieces 4 mm long) into the s.c. space between the shoulder blades. Experimental groups of 10 mice were given daily i.p. injections of 1 or 2 mg (when not otherwise specified) of the compounds (in 0.10 ml H₂O), starting 1 hr after tumor inoculation. Control groups of 10 mice were kept untreated. Animals of both control and experimental groups were weighed daily in batches of 10. Fourteen days later, all the controls and experimental animals were sacrificed by cervical dislocation. Tumors and spleens were excised and weighed in mg.

Change in carcass weight was determined by the equation:

$$Wc\% = \frac{(bw^f - tw - bw^i) \times 100}{bw^i}$$

in which *Wc* is weight change; *bw^f* is total weight on Day 14; *tw* is tumor weight; *bwⁱ* is body weight on Day 1.

Cell Culture Test. The compounds were evaluated for their effects on the growth of cells in suspension cultures. Compounds were present continuously in the growth medium, and cells were counted daily (14). Fibrosarcoma cells

were grown from an explant of the tumor used; the murine cells transformed by Friend leukemia virus, called erythro-leukemic cells, were the same as those used previously (14).

RESULTS

Results are summarized in Table 1. Treatment with modified dextran 1 (1 mg/day/mouse) resulted in a mild inhibition of tumor growth (26%) and increases both in body and spleen weight. Administration of aniline-dextran 2 (1 mg/day/mouse) led to a more substantial inhibition (37%) with a similar increase in the size of the spleens. The small-molecular counterpart of the aniline-dextran 2, Compound 3, had a moderate antitumor effect (29%) but with some toxicity (a lesser increase in carcass weight, and 1 animal dead on Day 13). Two different preparations of the sulfhydryl-dextrans were tested. Preparation 4, at a daily dose of 2 mg, inhibited tumor growth by 58%. The compound had no toxic effects, animals gained weight, and spleen size increased only moderately. Comparison with the data on aniline-dextran 2, which served for the preparation of sulfhydryl-dextran 4 and thus had the same degree of substitution, shows that the introduction of sulfhydryl groups does indeed increase the antitumor potency. Sulfhydryl-dextran 5, which has a higher degree of substitution than sulfhydryl-dextran 4 when tested at daily doses of 1 mg, inhibited tumor size by 44%; at the daily dose of 2 mg, inhibition was 74%. An increase of spleen size was observed in both instances, and even at the higher dose, there was a substantial gain in carcass weight (+5.4% versus 5.8% in the untreated controls). Antitumor effects of sulfhydryl-dextrans 4 and 5 are not only dose dependent but also proportionate to the amount of substituents with sulfhydryl groups, since Compound 5, which has the higher degree of substitution, is more active at the same dosage than Compound 4. The small-molecular counterpart, 6, of sulfhydryl-dextrans 4 and 5 had no antitumor effect. Neither of the small-molecular compounds, Compounds 3 and 6, had any significant effect on spleen size.

Mercury-dextran 7 had completely different effects and resulted in considerable increase in tumor size (61%). There was some increase in carcass weight (1.2 versus 5.8% in the untreated controls), an increase in spleen size, and no animal deaths.

The above-described compounds were also tested *in vitro* on cells which were grown from the explant of the studied fibrosarcoma. The aniline-dextran 2 and sulfhydryl-dextran 5, which retarded growth of fibrosarcoma in animals, were without effects on the growth of the fibrosarcoma cells *in vitro* even at the highest studied concentration (Chart 2). Mercury-dextran 7 at the same concentration (1 mg/ml) was clearly toxic, while at a concentration of 0.1 mg/ml it had only slight effects on cell growth. Effects of these compounds on growth of erythroleukemic cells were similar (data not shown); substituted dextrans 2 and 5 were without effect at a concentration of 1 mg/ml, while mercury-dextran 7 was still toxic at 0.1 mg/ml and nontoxic at 0.01 mg/ml.

The sulfhydryl-dextran 4 was also tested on the adenocarcinoma in syngeneic mice. At the daily dose of 2 mg, Compound 4 inhibited growth of the tumor by 65% (tumor

Table 1
Effects of substituted dextran and small-molecular-weight analogs on the growth of fibrosarcoma in syngeneic male C3Hf/Sed mice

Compound	Dosage (mg/day/mouse)	Tumor weight			Car-cass wt change (%)	Wt of spleen/normal spleens ratio
		Treated (mg)	Control (mg)	Change (%)		
1	1	555 ± 274 ^a	755 ± 298 ^a	-26	+ .41	1.8
2	1	487 ± 185	779 ± 135	-37	+6.8	1.6
3	0.08	166 ± 77	234 ± 99	-29	+1.4	1.2
4	2	327 ± 131	779 ± 135	-58	+6.1	1.7
5	1	189 ± 131	336 ± 164	-44	+9.5	1.6
5	2	178 ± 95	686 ± 132	-74	+5.4	3.0
6	0.12	243 ± 84	234 ± 99	+4	+4.2	1.2
7	1	235 ± 45	146 ± 15	+61	+1.2	2.8
Untreated				0	+5.8	1.4

^a Mean ± S.D.; 10 mice/experiment.

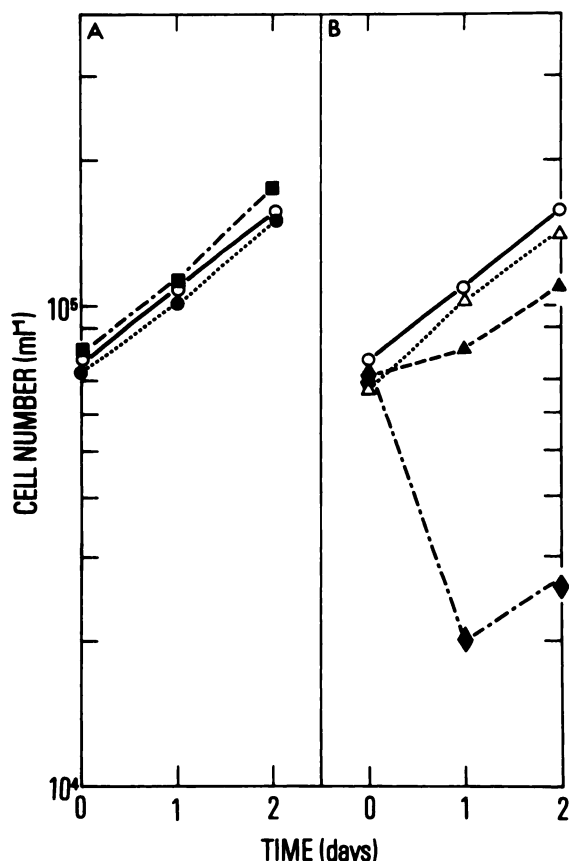


Chart 2. Growth of fibrosarcoma cells *in vitro* in the presence of substituted dextrans. Left: ○, control; ●, anilin dextran 2 (1 mg/ml); ■, sulfhydryl-dextran 5 (1 mg/ml). Right: ○, control; △, mercury-dextran 7 (0.01 mg/ml); ▲, 0.1 mg/ml; ◆, 1 mg/ml.

weights: treated, 125 ± 49 (S.D.) mg; controls, 353 ± 211 mg with 10 mice/experiment).

DISCUSSION

Substitution of dextran with electroneutral substituents can lead to compounds with considerable biological potency.

There is a sharp contrast in effects, which these macromolecules have, depending on whether tumor growth inhibition is studied *in vitro* or *in vivo*. Mercury-dextran 7

inhibited fibrosarcoma growth *in vitro*, while it promoted the growth of the same cells in the animals. Sulfhydryl-dextrans 4 and 5 and aniline-dextran 2 did not interfere with the growth of fibrosarcoma *in vitro* but retarded the growth of these cells in animals. Consequently, the effects of all of these compounds on tumor growth cannot be a direct one. The antitumor action may be due to the effects that these compounds exert on cells, which comprise a natural system of defense against tumors.

While this defense system has not been identified, the observed increase in spleen size, lack of general toxicity, and the comparison with these data on the other synthetic macromolecules (9, 13) suggest that the effects of substituted dextrans might be mediated through the reticuloendothelial system and macrophages. Possibly, the mercapto-dextrans 4 and 5 and aniline-dextran 2 can activate these cells to heightened tumoricidal activity, whereas the mercury-dextran 7 is presumably toxic to these cells.

The sulfhydryl-dextrans 4 and 5 and the mercury-dextran 7 have in common the ability to bind to the accessible sulfhydryl groups on membrane and serum proteins. Mercury-dextran 7 reacts by ligand exchange, yielding dextran-mercury-sulfur protein complexes; sulfhydryl-dextrans 4 and 5 form dextran-sulfur-sulfur-protein complexes. Nevertheless, these 2 groups of compounds have opposing effects on the growth of tumors. Another interesting observation is the lack of toxicity of the aniline-dextran 2 in spite of the fact that this macromolecule contains a toxic aniline moiety. Similar observations that the toxicity of agents decrease after attachment to dextran were made previously, e.g., on proflavine (14) or daunomycin (2).

In the design of macromolecular drugs with antitumor potency, it is usually assumed that endocytosis of foreign macromolecules is more active in tumor cells and that some differential toxic effects could be achieved on that basis. The results obtained in the study of mercury-dextran 7 show that such assumptions, while obviously correct in many instances (2, 6), may not be generally applicable. The study of a synthetic vinyl polymer which contains sulfonamide groups has previously led to a similar conclusion (15).

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